Pru p 3 mutants exhibit low IgE- binding capacity: a good strategy for specific peach immunotherapy

Gómez-Casado Cristina[1], Tordesillas Leticia[1], Cuesta-Herranz Javier[2], Gamboa Pedro[3], Palacín Arantxa[1], Díaz-Perales Araceli[1]

1 CBGP (UPM-INIA), Madrid (Spain), 2 Fundación Jiménez Díaz, Madrid (Spain), 3 Hospital de Basurto, Bilbao (Spain)

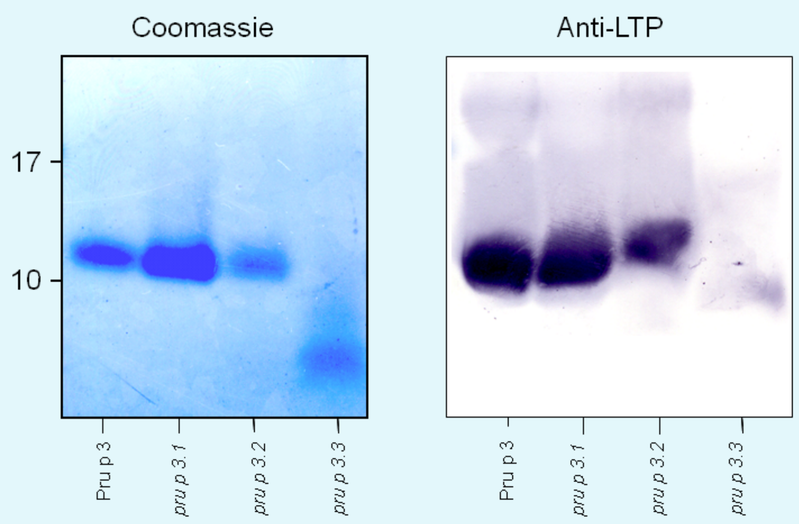
INTRODUCTION

Treatment of food allergy consists of the avoidance of the specific allergenic food. However, the possibility of cross-reactivity with other food sources makes this practice sometimes ineffective. The use of hypoallergenic molecules with the ability to stimulate T cells may be a promising tool for specific immunotherapy.

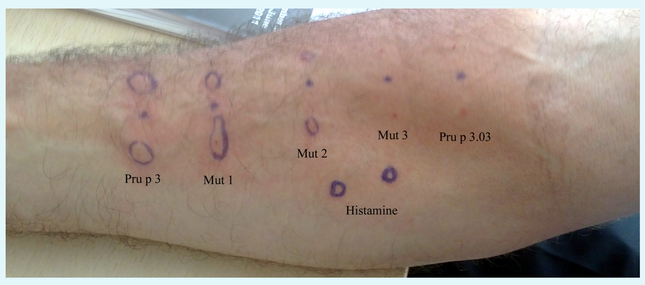
MATERIAL AND METHODS

The aim was to produce hypoallergenic Pru p 3, peach LTP, mutants by site-directed mutagenesis, in residues involved in B-epitopes.

Three Pru p 3 mutants were produced in *Pichia pastoris* and purified by chromatographic methods.

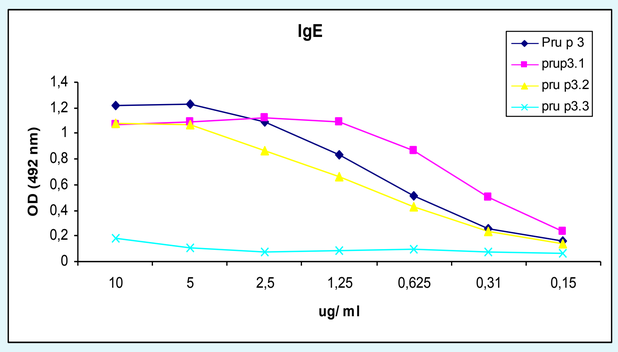


The IgE, IgG1 and IgG4-binding capacities of the three of them were tested by direct and inhibition ELISA assays with patients' sera. Their allergenic capacity was evaluated by skin prick test. The T-cell response induced by these antigens was compared with that of the wild type.



RESULTS AND CONCLUSIONS

IgE binding capacities of *pru p 3.2* and *pru p3.3* were reduced in most tested sera (9-96%), and confirmed by inhibition ELISA assays with a serum pool from peach-allergic patients.



Reduction

pru p 3.1 25% (range 1-59%)

pru p 3.2 24% (range 0-46%)

pru p 3.3 65% (range 32-93%)

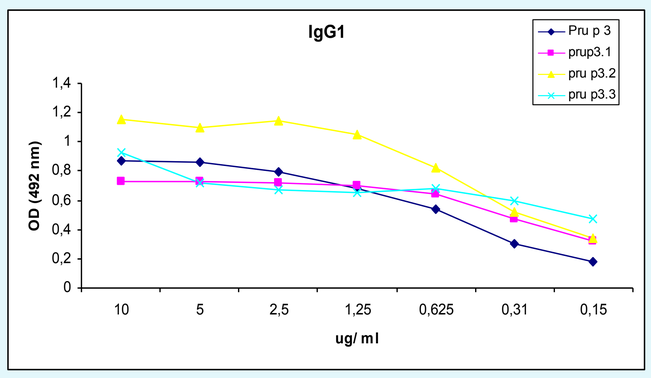
By contrast, IgG1 epitopes were mostly conserved.

Reduction

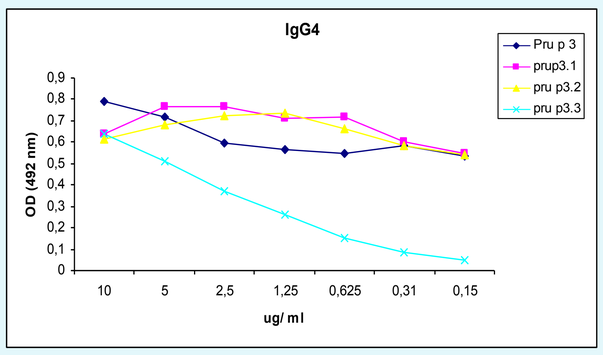
pru p 3.1 21% (range 2-43%)

pru p 3.2 1% (range 0-4%)

pru p 3.3 29% (range 0-62%)



Regarding IgG4 epitopes, Mut*3* showed decreased binding capacity (58-97%).



Reduction

pru p 3.1 12% (range 0-51%)

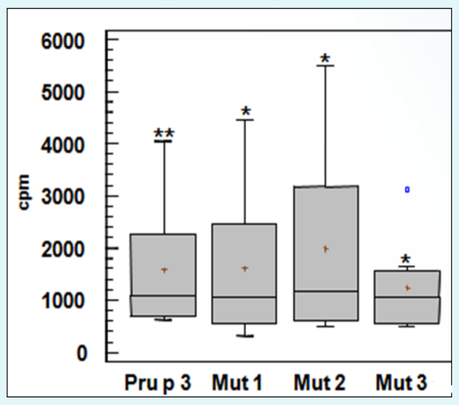
pru p 3.2 19% (range 0-57%)

pru p 3.3 72% (range 58-97%)

The *in vivo* allergic response was significantly lower for *pru p 3.1* and *pru p 3.2*, and negative for *pru p 3.3*, confirming the lack of IgE binding by the ELISA assays.

Patient	Age (years)	Sex	Skin Prick Test (mm²)*				
			Pru p 3	Mut 1	Mut 2	Mut 3	Pru p 3.03
1	38	F	57	29	18	0	0
2	55	M	15	13	6	0	0
3	39	M	47	12	10	0	0
4	45	F	43	14	11	0	0
5	43	M	17	3	0	0	0

*SPT>9mm² positive response



T-cell activation capacities were similar for the natural protein and the mutants. All these data suggest that these molecules could be useful for specific peach immunotherapy.